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Assessment of Adolescent Neurotoxicity: Rationale and Methodological Considerations:

An Introduction to the Special Issue on "Risk of neurobehavioral toxicity in adolescence"

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Abstract

This introduction to the special issue of Neurotoxicology and Teratology on "Risk of neurobehavioral toxicity in adolescence" begins by broadly considering the ontogeny and phylogeny of adolescence, and the potential value of animal models of adolescence. Major findings from the emerging neuroscience of adolescence are then highlighted to establish the importance of studies of adolescent neurotoxicity. A variety of methodological issues that are of particular relevance to adolescent exposures are then discussed. These include consideration of pharmacokinetic factors, inclusion of other-aged comparison group(s), and issues involving timing, route of administration, and exposure-induced alterations in growth rate. Despite such methodological challenges, research to determine whether adolescence is a time of increased vulnerability (or greater resiliency) to specific drugs and environmental toxicants is progressing rapidly, as exemplified by the work presented in the articles of this special issue.

Keywords

adolescence; neurotoxicity; behavior; animal models; brain sculpting; pharmacokinetics; drug metabolism; body weight; methodology

Most of what we know about neural and behavioral consequences of developmental exposure to drugs and other chemicals is based on exposures during the prenatal and early postnatal period, with little emphasis on exposure periods that subsume adolescence. With the increasing recognition that adolescence is a time of considerable neural restructuring and sculpting of the brain (for review, see Spear, 2000), there likewise has been a growing interest in assessing whether this developmental transition is a vulnerable period for neurotoxicity. This special issue is designed to highlight research examining "the question of whether adolescence is a…. (time) of enhanced neurobehavioral toxic risk associated with exposure to drugs of abuse, therapeutic drugs, hormones and environmental toxicants." By presenting examples of the emerging research in this area, the goal of the special issue is to encourage additional high quality work in this area, and to draw attention to Neurotoxicology and Teratology as an outlet for this research.

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To set the stage for the research presented in the special issue, this introduction will begin by considering the ontogeny and phylogeny of adolescence, and the value and limitations of animal models of adolescence. Major findings from the emerging neuroscience of adolescence will then be highlighted to establish the importance of studies of adolescent neurotoxicity. Following this foundation, methodological considerations of particular importance to work involving adolescent exposure periods will then be addressed.

Adolescence: ontogeny, phylogeny, and the use of animal models

Adolescence consists of a series of characteristic alterations that are seen during the transition from immaturity/dependence to maturity/independence. Among the physiological transitions seen at some point during this broad developmental period are puberty and its concomitant hormonal and physiological changes, along with a considerable growth spurt. As discussed later, the brain of the adolescent also undergoes pronounced sculpting and modification. Adolescence is likewise characterized by expression of a number of typical behavioral features, such as increased novelty seeking and risk taking (Irwin, 1989;Trimpop et al, 1999) and a shift in social affiliation towards more peer-based social interactions (La Greca et al., 2001). It is during adolescence as well that many individuals begin experimenting with alcohol and other drugs. Alcohol use becomes normative, with per episode alcohol intakes among adolescent drinkers averaging about twice those of adult drinkers (SAMHSA, 2004).

The developmental stage of adolescence is not uniquely human. Developing organisms from other species likewise undergo adolescent-typical transitions that include pubertal changes and a growth-spurt, along with expression of certain adolescent-typical behavioral patterns (see Spear, 2000, for review). For instance, even when considering a simple animal model of adolescence in the rat, animals undergoing this transition exhibit more risk taking, novelty-seeking, and peer-directed social interactions than adults (e.g., Douglas et al, 2003, 2004;Stansfield & Kirstein, 2006). Adolescent rats also voluntarily consume 2–3 times more alcohol relative to their body weight than adults under a number of circumstances (Brunell & Spear, 2005;Doremus et al, 2005) and find social peers (Douglas et al, 2004), novelty (Douglas et al, 2003) and nicotine (Vastola et al, 2002) more rewarding than their adult counterparts.

Similarities across species in adolescent-typical physiological and behavioral characteristics are consistent with the notion that adolescence has been a highly conserved developmental stage during evolution (Savin-Williams & Weisfeld, 1989), with a number of specific adolescent-typical behavior patterns postulated to confer adaptive significance (see Spear, 2000, for discussion). Across-species similarities in physiological and behavioral attributes of adolescence provide reasonable face and construct validity for the use of animal models to study potential neurotoxic effects during adolescence. That is not to imply, however, that all consequences of human drug or toxicant exposures can be modeled in non-human animals. Of course, no other species demonstrates the full complexity of human brain and behavior evident during adolescence (or at any stage of life, for that matter). It is only through consideration of the specific neurotoxic features targeted for examination that it can be determined whether they can be examined effectively using animal models, and what animal model would be most appropriate.

A critical issue when assessing consequences of drug/toxicant exposure during adolescence is the timing of the exposure period. This is often not straightforward, in that there is no single event that signals the onset or offset of adolescence in any species, with notable individual differences in timing driven in part by genetic differences, gender, and nutrition, along with other environmental factors (e.g., Frisch, 1984;Kennedy & Mitra, 1963). For example, although the adolescent period in humans has been considered by a variety of developmental researchers to typically span the age range from 12–18, some emerging signs of adolescence

may begin as early as 8–10 (especially in females), with other characteristic features lasting until 25 years or later (Baumrind, 1987;Parker, 1991). The precise timing of adolescence defies absolute categorization in other species as well. In rats, the age range from 28–42 days postnatal has been conservatively classified as adolescence, based on timing of age-specific behavioral changes, neural changes in brain, puberty, and the growth spurt. Yet, again some harbingers of adolescence may begin in females as early as 23 or so days postnatally, with some residual signs perhaps lasting until 55 days or so in males (see Spear, 2000). Thus, to ensure that an exposure period blankets the entire adolescent period in rats of both sexes, it might be necessary to begin shortly after the conventional age of weaning and continue until at least 55 days or so. Depending on the research questions under investigation, more restricted exposure periods may sometimes prove useful for revealing particular portions of the adolescent period as times of enhanced neurotoxic vulnerability relative to other adolescent intervals (e.g., see work by Abreu-Villaca et al, 2003a,b;Adriani et al, 2002,2003,2004). Unfortunately, some research purported to be focused on adolescence tests animals outside of such age ranges, with often little evidence provided to justify the chosen ages. At this early stage in the study of adolescent neurotoxicity, it seems critical to carefully consider the age range chosen for investigation, and to provide clear justification for that age span.

The neuroscience of adolescence

Despite some early evidence for notable ontogenetic dissociations in behavior and psychopharmacological sensitivity during adolescence (e.g., Spear & Brake, 1983), it has only been within the last decade or so that studies of neural development have begun to include a focus on adolescence. The increasing emphasis on research investigating brain maturation during adolescence is evident both in studies using animal models and in work with developing humans, the latter of which has been aided considerably by rapid advances in imaging technology that permit non-invasive assessment of human brains without the use of radioactivity. Both the imaging work in humans and the often more detailed neuroanatomical assessments emerging from studies with laboratory animals have revealed a surprising degree of neural sculpting during adolescence, with notable similarities in the brain regions affected often emerging across a variety of species (see Spear, 2000, in press).

Prominent among the neural alterations seen during adolescence is a substantial culling of synaptic connections, with close to 50% of the synaptic connections lost in some cortical regions (Rakic et al, 1994). Synaptic pruning is more pronounced in prefrontal cortex (PFC) and other neocortical regions than subcortical areas (Rakic et al, 1994). Included among the synapses undergoing particularly notable pruning during adolescence are those providing excitatory input to the neocortex (Bourgeois et al, 1994) as well as synapses contributing to reverberating circuits within particular cortical regions (Woo et al, 1997). This ontogenetic reduction in excitatory input to cortex and connectivity within reverberating circuitry could contribute to the reduction and refinement of brain effort during adolescence. Indeed, adolescence is associated with a considerable decline in brain energy utilization in humans (Chugani, 1996) and other species (e.g., rats: Tyler & van Harreveld, 1942), with the high rates of blood flow and the elevated rates of oxygen and glucose utilization seen during childhood gradually declining through adolescence to reach the lower rates of energy consumption characteristic of the adult brain.

Progressive myelination of axons results in considerable developmental increases in cortical white matter through adolescence and into adulthood; myelination serves to accelerate information flow along axons, and has been presumed to increase overall speed of information processing within the brain (e.g., Villablanca et al, 2000;Sowell et al, 2003). Although less prominent and consistent than developmental increases in white matter, ontogenetic declines in volume of gray matter (cellular regions) are seen in regions such as the frontal cortex (Giedd

et al, 1999;Rapoport et al, 1999), although some developmental increases in gray matter volume are reported in other areas (e.g., amygdala and hippocampus - Giedd et al, 1997). Declines in relative volume of gray matter in particular brain regions may reflect both the culling of synapses as well as ontogenetic increases in white matter, with overall cerebral volume remaining about the same from 5 years of age onward in humans (Giedd et al, 1996).

Among the brain regions showing particularly marked ontogenetic alterations during adolescence is the PFC, with this region showing considerable synaptic culling and a prominent loss of excitatory input (Rakic et al, 1994;Bourgeois et al, 1994). Developmental studies using functional magnetic resonance imaging (fMRI) have revealed notable ontogenetic changes in PFC activation during performance of cognitive tasks thought to index various components of executive function (e.g., response inhibition, working memory, attention)(e.g., Casey et al, 1998,2000;Luna et al, 2001;Paus, 2005). Such fMRI studies have sometimes revealed declines in subcortical activation between adolescence and adulthood that are the inverse of the ontogenetic increases in activation seen in certain frontal regions (Rubia et al, 2000). Indeed, although substantial emphasis has been placed on alterations in PFC and other regions of neocortex during adolescence, certain subcortical regions also undergo considerable remodeling during adolescence as well - especially those regions that form part of an interconnecting network of circuitry with the PFC - e.g., the amygdala and extended amygdala, and other dopamine (DA) mesocorticolimbic terminal regions. For instance, projections from the amygdala to PFC continue to be elaborated through adolescence (Cunningham et al, 2002). The amygdala of the adolescent also shows a different pattern of stress-induced activation than the adult (Kellogg et al, 1998), with fMRI data as well often revealing ontogenetic differences in amygdalar activation to emotional stimuli (faces) between adolescents and adults (e.g., Killgore et al, 2001;Thomas et al, 2001; although see also Pine et al, 2001).

Among the numerous alterations seen in mesocorticolimbic brain regions during adolescence are regionally specific ontogenetic alterations in patterns of DA production and utilization, with estimates of DA synthesis and turnover in PFC being higher early in adolescence than later in adolescence and in adulthood, whereas DA synthesis and/or turnover estimates in nucleus accumbens and striatum conversely are lower earlier than late in adolescence (e.g.,Teicher et al, 1993;Andersen et al, 1997, although see also Leslie et al, 1991). Stressors would likely exacerbate the shift in DA balance toward even greater mesocortical than mesolimbic/striatal DA activity during early adolescence, given the greater sensitivity of the mesocortical DA projection system to activation by stressors (Dunn, 1988). These adolescent-typical alterations in neurocircuitry involving the extended amygdala and related forebrain DA terminal regions are likely to be of functional significance for the adolescent. DA projections to mesolimbic brain regions and the PFC form part of the circuitry critical for modulating risk taking, novelty seeking, and social behaviors (e.g., Le Moal & Simon, 1991), and for attaching motivational relevance to natural rewards (such as social stimuli, novelty, food) as well as alcohol and other drugs of abuse (e.g., Robinson & Berridge, 2003).

As illustrated by these examples, the brain undergoes considerable sculpting and remodeling during adolescence (see Spear, 2000, for further review). During this transformation, the brain must support critical adolescent behaviors and the marked physiological and hormonal transformations of this age period, while also serving as the substrate for the eventual emergence of the mature brain. This adolescent-typical remodeling of brain could provide a relatively delayed window of opportunity to sculpt the brain to match the environmental circumstances encountered by the organism as it approaches maturity (see Andersen, 2003). Indeed, the greater capacity for synaptic remodeling seen developmentally through adolescence diminishes markedly following adolescence (Gan et al, 2003).

The question remains, however, as to whether the neural alterations occurring during this developmental phase are unusually sensitive to disruption by specific drugs or toxicants, or whether this remodeling reflects a window of opportunity for unusual plasticity and recovery. It seems likely that the answer may vary with test substance, duration and timing of exposure, and the target measures being examined. On the one hand, evidence is beginning to emerge to support the suggestion that, for certain drugs, adolescence may be a time of enhanced neurotoxic sensitivity relative to adulthood (e.g., for reviews see Slotkin, 2002;Smith, 2003). Yet, there are also some intriguing signs that under certain specific circumstances, the adolescent brain may perhaps show unsuspected resiliency to neurotoxic insult. For instance, there is a recent report that changes in mRNA expression patterns seen following chronic access to alcohol in adulthood were not observed following a comparable period of access during adolescence (Falco et al, 2006). Substantially more research is needed to determine the generalizability of these reports of adolescent-specific vulnerabilities and resiliencies, and to characterize under what exposure and test circumstances these effects are observed. There are a number of methodological challenges inherent in such work, as outlined in the next section.

Methodological considerations in studies of adolescent neurotoxicity

When conducting research to examine adolescent neurotoxicity, a number of issues arise that are often less relevant to exposures at other points in the lifespan. Some of these potential difficulties can be relatively easily accommodated when developing the experimental design or methods to be used in particular studies. Other issues may be difficult to address at the design level, but are important to consider when interpreting and drawing conclusions from adolescent exposure data.

Other-aged comparison group(s)

Determining that a given drug or environmental toxicant produces lasting effects following chronic exposure during adolescence may not be particularly meaningful without one or more comparison groups exposed to an equivalent amount of the substance at another age. That is, in the absence of another-aged comparison group(s), even if long-term consequences of adolescent exposure are observed, those effects could reflect either an enhanced, equivalent or reduced neurotoxic potential during adolescence relative to exposure at maturity or some other point in the lifespan. Hence, to draw conclusions regarding whether neurotoxic risk to a particular substance is increased during adolescence, outcomes of adolescent exposures must be compared with equivalent exposures conducted during some other phase(s) of the lifespan. At first glance, this approach seems to vary from prenatal studies in neurotoxicology where a single chronic exposure period may be targeted. Yet, prenatal studies inherently contain an other-aged comparison group: the pregnant female That is, consequences of prenatal exposure to a test substance can only be assessed at exposure levels that, by necessity, are not toxic to the dam.

Issues of timing

Research in prenatal toxicology has revealed that the timing of the prenatal exposure may critically influence the findings obtained, with as little as a one day difference in drug exposure sometimes resulting in markedly different outcomes (e.g., see Vorhees, 1987). The same may prove to be true within the adolescent period as well. Although there is little if any research that has directly examined variations in neurotoxic sensitivity within the adolescent period, there is evidence that neural transformations and pharmacological sensitivities vary considerably from early to late adolescence. For instance, based on neurochemical evidence it has been postulated that there is a shift in balance within the mesocorticolimbic dopamine system during adolescence (Spear, 2000;Andersen, 2003), with greater mesocortical DA activity early in adolescence preceding a shift to greater DA activity in mesolimbic regions

such as the nucleus accumbens later in adolescence (see Spear, 2000, for review). Early adolescence has been shown to be a time of particular marked adolescent-typical alterations in psychopharmacological sensitivity to ethanol (Varlinskaya & Spear, 2004;2006) and nicotine (Adriani et al, 2002;Abreu-Villaca et al, 2003a,b;Adriani et al, 2003; but see also Adriani et al, 2004).

When considering timing of neurotoxic exposures within adolescence, it is also important to consider sex differences and the timing of puberty within the broader adolescent period. Pubertal transitions often occur relatively early during adolescence, with females tending to mature more rapidly and proceed through puberty earlier than males in humans (Van Vliet, 1991;Wheeler, 1991) and other species (e.g., Weisfeld, 1979;Odell, 1990). To the extent that exposure to a particular drug/toxicant influences pubertal-related neural systems or other neural alterations whose pacing differs between males and females, sex differences in timing of developmental vulnerabilities to neurotoxic effects may emerge. Thus, different conclusions regarding the relative sensitivity of males versus females to neurotoxic effects during adolescence could emerge depending on when that exposure period occurred within the broader adolescent period.

One design issue that plagues research that compares outcomes following exposures at different ontogenetic stages regards the potentially confounding variables of time and age. That is, when animals are exposed to a substance at different ages, one must decide whether to test animals following the same post-exposure recovery interval, hence confounding age at the time of testing across exposure groups, or to test animals at the same ontogenetic age, with a resultant confound across groups in the time from exposure to test. Recovery time and chronological age are both critical variables. Certainly in the literature examining consequences of withdrawal from chronic drug exposure in adulthood, the interval between drug termination and test has been shown to crucially influence measures of neurobehavioral function (e.g., Bienkowski et al, 2004;Lu et al, 2004). Chronological age, even within adulthood, has likewise been shown to impact various neural and behavioral measures (e.g., Benes, 1994). The decision of post-exposure-to-examination interval is not a trivial one. Consider, for example, the regionally- and time-specific nicotinergic receptor up regulation that emerges following nicotine exposure in adolescence versus adulthood (Trauth et al, 1999). If this work had instead focused on only a single brain region and sacrifice interval, the data obtained could have supported the conclusion that nicotine exposure in adolescence induces more receptor upregulation than in adulthood, or less up regulation, or even equivalent upregulation at both ages - depending on the specific brain region and sacrifice interval chosen for examination.

There are a number of possible strategies for addressing the potential confound of age versus exposure-to-test interval in studies comparing neurotoxicity in adolescents and adults. One approach is to test two different groups of developmentally exposed animals - one following the same post-exposure recovery interval as adult-exposed animals, and the other at the same chronological age as the adult-exposed animals. Whether this approach justifies its costs (both financially and in terms of animal resources) may depend on project goals and stage of investigation of the developmental neurotoxicity of the substances. When such a design is not used, conclusions should be tempered by recognition that the variable chosen for confounding may have contributed to the effects observed.

Pharmacokinetic issues

One challenge when attempting to compare outcomes of drug/toxicant exposures conducted in adolescence with those at other ages is pharmacokinetic. It is difficult to determine if adolescents are at increased risk relative to more mature animals for some particular consequence in the absence of pharmacokinetic information. For instance, consider a hypothetical study where adolescents were found to develop marked neurotoxicity to a test

substance at an exposure level that did not induce neurotoxicity in adults. While it would be tempting to conclude from such findings that adolescents are more sensitive to neurotoxic consequences of the substance than adults, this conclusion could be premature. For instance, it could be the case that metabolism of the test substance into inactive metabolites occurs substantially more slowly in adolescents than adults, resulting in brain levels of the test substance that are notably higher among the adolescents, and that it is these elevated brain levels that are responsible for the greater neurotoxicity. Administration of equivalent doses across age does not necessarily mean that exposure levels are equivalent. Unfortunately, studies comparing brain (or even blood) levels of drugs/toxicants following exposures at different stages of ontogeny are infrequent.

There is evidence that under a number of circumstances adolescents tend to metabolize drugs and other substances more rapidly than adults. Resting metabolic rate is inversely related to body weight (more accurately, body surface area - Schmidt-Nielsen, 1972), and hence is higher during adolescence than in adulthood (Iossa et al, 1999). Rates of metabolism of a variety of drugs tend to be slightly elevated as well in adolescents relative to adults (e.g., McCarthy et al, 2004). This may be related in part to the greater hepatic capacity and more efficient renal mechanisms relative to their size that juveniles have when compared to adults; both of these factors could contribute to faster elimination of drugs using these pathways by juveniles than adults (Geller, 1991).

For test substances that distribute in body water, distributional factors also would tend to support lower blood levels in organisms prior to maturity, given that percentage of body water (and hence relative volume of distribution) is inversely related to body weight and hence declines ontogenetically (Wiberg et al, 1971;York, 1983). Basing drug doses on body weight may also bias for relatively larger exposure levels in adults than immature animals. As animals grow, increases in body weight are more marked than increases in body surface area, although the latter is more closely related to overall metabolic rate than body weight (e.g., Schmidt-Nielsen, 1972). Hence, as size increases ontogenetically, drug doses based on body weight rise faster than if they were based on surface area, amplifying dose effects relative to overall metabolic rate in larger (e.g., adult) animals relative to their smaller (e.g., adolescent) counterparts.

Although age differences in drug levels exerted by these pharmacokinetic factors in conglomerate are often surprisingly slight (see discussion below), when significant differences emerge, they tend to produce lower functional drug concentrations in adolescents than adults following administration of the same exposure dose. Under such circumstances, any greater sensitivity of adolescents to neurotoxic effects would seemingly occur despite the tendency for lower exposure levels. Indeed, this strategy of determining whether adolescents display enhanced neurotoxicity relative to adults despite measured (or assumed) lower functional drug concentrations among the adolescents seems to implicitly, if not explicitly, underlie much of the research in this area. This strategy relies on at least two assumptions. One is that neurotoxicity is monotonically related to dose; should dose and neurotoxicity be related by a U- or J-shaped function (e.g., Calabrese & Baldwin, 2002), though, greater damage could emerge at lower than higher neural concentrations of the drug. Another necessary assumption with this approach is that metabolites of the drug are inactive, or at least less active than the parent compound; this is not necessarily the case with all drugs (e.g., consider the active nicotine metabolite, cotinine - see Slotkin, 2002, for discussion).

Pharmacokinetic issues during ontogeny have been examined most systematically with ethanol. Rates of ethanol metabolism increase developmentally to reach a peak or plateau during adolescence (Silveri & Spear, 2000), with adolescent animals sometimes (Holstedt et al, 1977; Brasser & Spear, 2002) but not consistently (Kelley et al, 1987;Silveri & Spear,

2000) observed to have slightly but significantly faster rates of ethanol metabolism than mature animals. Any slight difference in ethanol metabolism between adolescents and adults rarely results in different blood alcohol levels following injection of low-to-moderate doses of ethanol (e.g., Varlinskaya & Spear, 2002), however, and is generally insufficient to account for the attenuated sensitivity shown by adolescents to many acute effects of ethanol (e.g., see Silveri & Spear, 2000). There are also some ontogenetic differences in ethanol distribution rates that emerge in male rats following fairly high doses of ethanol, with the distribution phase taking longer in adult males than adolescent animals (and adult females). As a consequence, clearance is delayed, with the net result that the overall amount of ethanol to which the adult males are exposed (i.e., area-under-the-curve) is greater relative to the total exposure levels of adolescent animals and adult females (Varlinskaya & Spear, 2004).

Similar findings have been reported with nicotine. At infusion rates of 3–4 mg/kg/day for adolescents and 5 mg/kg/day for adults, Slotkin (2002) reported that adults had proportionally greater plasma levels of both nicotine (3–4 fold greater) and the nicotine metabolite, cotinine (2–3 fold greater) than adolescents. Evidence for a sex difference also emerged among the adolescents, with males and females having similar levels of both nicotine and cotinine despite the lower nicotine infusion rate reached in males (3.0 mg/kg/day) relative to females (3.6 mg/kg/day) when samples were collected at the end of the infusion period (Slotkin, 2002). Thus, adolescents appear to metabolize nicotine somewhat more rapidly than adults, with female adolescents tending to metabolize nicotine even more rapidly than their male counterparts.

There are a few other scattered reports of developmental differences in pharmacokinetics. Brain levels of amphetamine were found to be lower in pre-adolescent (postnatal day 25 [P25]) rats than adolescent (P35), post-adolescent (P45) and adult rats, and with a trend for lower brain amphetamine levels among the adolescent than the older animals as well (see Spear & Brake, 1983). Likewise, 15 minutes following acute administration of cocaine, adolescent mice have been reported to have lower levels of cocaine and higher levels of the cocaine metabolite benzoylecgonine (BZE) than adults (McCarthy et al, 2004). Blood levels of the selective serotonin reuptake inhibitor (SSRI) fluoxetine tend to decline more rapidly in adolescents than adults, with a trend for lower levels of norfluoxetine, an active metabolite of fluoxetine, among adolescents as well (Brunell & Spear, in preparation). Human adolescents have been reported to have a shorter half-life for the SSRI paroxetine than adults (Findling et al, 1999), although no apparent age differences emerged with another SSRI, sertraline (Axelson et al, 2002). Thus, across a number of different classes of drugs, when differences in blood or brain levels in the drug emerge between adolescents and adults, lower levels tend to be present among the adolescents. This generalization is likely to have exceptions, however, and it should not be assumed that similar ontogenetic findings necessarily would emerge when examining other drugs/toxicants - particularly when the pharmacokinetic picture is complicated by repeated exposures.

Indeed, pharmacokinetics of drug metabolism may change with repeated exposure to the drug, and may do so differentially across age. For instance, whereas both adolescent and adult rats develop functional tolerance to ethanol-induced social suppression when exposed chronically to ethanol, Varlinskaya and Spear (in press) have demonstrated that it was only in the adults that metabolic tolerance could have contributed to this effect. That is, adults challenged with ethanol following a period of chronic ethanol exposure had lower blood ethanol levels than adults who had not previously been exposed to ethanol, whereas no difference in ethanol levels following challenge emerged between chronically ethanol-exposed and control adolescents (Varlinskaya & Spear, in press). To the extent that metabolic tolerance develops more rapidly in mature animals than adolescents to other drug or environmental toxicants, over the course of the chronic exposure period this effect of repeated exposure would tend to diminish age differences in drug levels produced by other pharmacokinetic factors. Indeed, the lower levels

of cocaine and elevated levels of the cocaine metabolite BZE seen in adolescent relative to adult mice were only evident after acute exposure to cocaine, with no evidence of any age differences in these measures following chronic cocaine administration (McCarthy et al, 2004). Differential emergence of metabolic tolerance (or other pharmacokinetic adaptations) across age would make it challenging to equate total amount of chronic exposure at different ages, necessitating caution when interpreting across-age data.

Route of administration

Route of administration and its relevancy to typical human exposure routes is an important consideration at all exposure ages, although adolescent exposures can sometimes add additional complexities. For instance, when using osmotic minipumps to administer nicotine, mg/kg/day release rates change much more dramatically during the growth spurt of adolescence than during the typically more moderate rises in body weight characteristic in adulthood. If initial exposure rates are equated across age, much lower mg/kg/day exposures are evident by the end of the exposure period among adolescents than adults. An alternative approach is to load the minipumps so that initial release rates are higher in adolescents than adults to equate "area under the curve" (i.e., mean daily mg/kg exposure rates averaged over the total exposure period) (see Wilmouth & Spear, submitted).

When administering the drug orally through dietary administration or via voluntary oral self-administration, there is the complication that adolescents typically consume more food and fluids relative to their body weights than animals at other ages. Thus, when indexed in terms of kg of body weight, consumption of target substances will likely be greater in adolescence than adulthood, regardless of whether the target substance is placed in the diet or consumption is voluntary (e.g., 2-bottle tests). These elevated adolescent intakes, associated in large part with the elevated caloric demands of the considerable growth spurt of adolescence (e.g., Nance, 1983;Post & Kemper, 1993;Ganji & Betts, 1995), make it challenging to equate exposure levels across age when using oral consumption. While oral intake across age can sometimes be equated by varying concentration of the test substance to be used at each age (e.g., Silveri et al, 1999), in other instances the pharmacological properties of the test substance itself may help drive ontogenetic differences in consumption. For instance, the greater voluntary consumption of ethanol often seen among adolescent than adult rats is not merely a function of the hyperdipsia of adolescence or ethanol's caloric properties (Doremus et al, 2005) and may be driven in part by the attenuated sensitivity of adolescents to aversive effects of ethanol that may normally serve to limit intake (see Spear & Varlinskaya, 2005, for discussion).

Exposure-induced alterations in growth rate

Elevated caloric demands and the growth spurt of adolescence provide an additional challenge when assessing neurotoxic effects of adolescent exposures. Because of their enhanced caloric needs, minor perturbations in consummatory behavior induced by environmental conditions or toxicant exposure may have exaggerated effects on adolescents. For instance, in work to develop a food restriction schedule for adolescents, we initially gave adolescent rats the same grams of food as food-restricted adults and found that the adolescents, despite their smaller size (and hence proportionally greater g/kg food allocation), lost more weight than the adults (Vetter & Spear, unpublished observations). Under some circumstances, adolescents have been found to be more sensitive than adults to chronic stressors such as social stress, restraint, and isolate housing (Einon & Morgan, 1977;McGivern et al, 1996;Stone & Quartermain, 1998), stressor effects that may include an adolescent-specific suppression of food intake and weight gain (see Stone & Quartermain, 1998). Any reduction of weight gain during adolescence is likely to alter the pace of development, delaying puberty, extending the adolescent period, and perhaps altering the normal trajectory of developmental processes. Indeed, body weight or composition (i.e., proportion of body fat) is more strongly linked to the timing of puberty than

chronological age per se in species ranging from rodents (Kennedy & Mitra, 1963) to humans (Frisch, 1972,1984). Thus, when assessing effects of potential neurotoxic agents during development, exposure-related effects on body weight should be monitored. In cases where notable disruptions in weight gain are observed, it might ultimately prove necessary to include body weight controls (e.g., pair-fed animals) to dissect whether neurotoxic effects of adolescent exposures are a function of exposure to the substance per se or whether they arise indirectly though undernourishment. These issues have received little attention to date at this early stage of research in adolescent neurotoxicity.

Conclusions and questions for the future

Adolescence is a unique and highly conserved age period with characteristic behavioral and physiological features, including a marked remodeling of the brain. But is this remodeling of adolescent brain a time of increased or decreased vulnerability to drugs/toxicant exposures? Is the sculpting of the adolescent brain disrupted by exposure to neurotoxicants, resulting in the production of a different brain, and resulting in long-lasting alterations in neural functioning that are not evident following equivalent exposures to the less plastic adult brain? Or does this time of neural plasticity provide an enhanced opportunity for the nervous system to recover from neurotoxic insults delivered during adolescence, resulting in fewer long-term consequences than seen with comparable adult exposures? Could the time window of altered vulnerability or resilience vary with neural region and test substance? Despite numerous methodological challenges, research to answer these questions is evolving rapidly, as illustrated by the work presented in the articles in this special issue.

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